STIC-ILL (NLM) QC321, A47

From: Schmidt, Mary
Sent: Thursday, December 12, 2002 2:48 PM
To: STIC-ILL
Subject: last set of references 09/122,588

1635

4240061

VEGF references:

Thierry et al., Proceedings of the american association for cancer research annual meeting, 36, 0, p413, 1995.

Yung et al., neurology 48, 3 suppl., 2, pa22, 1997.

ke et al., proceedings of the american association for cancer research annual meeting, 37, 0, p60, 1996.

ellis eta l., surgery, nov. 1996, 120, 5, p871-8.

martiny-baron et al., current opinion in biotechnology, dec. 1995, 6, 6, p675-80.

warren et al., journal of clinical investigation, apr. 1995, 95, 4, p1789-97.

o-brien et al., cancer research , feb. 1, 1995, 55, 3, p510-3.

chaudhary et al., molecular biology of the cell, 7, suppl., p352a, 1996.

saleh et al, cancer research, 1-15-1996, 56, 2, 393-401.

claffey et al, cancer research, 1-1-1996, 56, 1, 172-81.

levy et al, j. of biological chem, 10-11-1996, 271, 41, p25492-7.

thanks,
Melissa
11e12 mailboxes

6447366

Afternoon Session

S ssion No. VI3
NEURO-ONCOLOGY: EXPERIMENTAL

M nday, April 14

3:45 PM – 5:00 PM

Room 112

Co-chairs: WR Shapiro, Phoenix, AZ
RG Wiley, Nashville, TN

VI3.001
3:45 PM

Molecular Modulation of Vascular Endothelial Growth Factor (VEGF) Expression in Glioma Cells by Ribozymes

W. K. Alfred Yung, LiDao Ke, XiaShan Chen, Peter A. Steck, Houston, TX, USA.

OBJECTIVE: To modulate the expression of VEGF in human glioma cells by RNA ribozymes.

BACKGROUND: VEGF, a potent angiogenic growth factor, has been shown to be overexpressed in human glioblastomas. It induces angiogenesis in a paracrine fashion since VEGF receptors are expressed only on endothelial cells. Ribozyme, a antisense RNA molecule with catalytic activity, is capable of cutting mRNA at specific sites, thus deplete the mRNA level for subsequent protein synthesis.

DESIGN/METHODS: Nineteen potential ribozyme cutting sites were identified in the human VEGF mRNA sequence. Two ribozyme motifs (RZmI, RZmII) that were designed to cut the VEGF mRNA in the common region of the three VEGF varients expressed in human gliomas were constructed. The effectiveness of the ribozymes were tested by several in vitro studies.

RESULTS: In vitro cell-free studies had demonstrated efficient digestion of a 309 bases VEGF RNA (RZt) template sequence in the predicted sites. RZmI and II were then cloned into pSG5 and pCEP4 vectors for transfection studies in U251 glioma cells. Western blot and ELISA analyses showed decreased level of VEGF protein in transfected cells. RT-PCR also showed decreased level of VEGF mRNA in transfected clones.

CONCLUSIONS: These preliminary results suggest that the ribozyme approach can be a potential molecular strategy to regulate the expression of VEGF in human gliomas.

Supported by: NCI CA51148, CA55261, and Gilliland Foundation.

VI3.002
4:00 PM

Adenovirus-mediated p16 Transfer Suppresses Glioma Invasion

Athanassios P. Kyritsis, Juan Fueyo, Candelaria Gomez-Manzano, Shravan K. Chintala, Boyapati Venkaiah, W.K. Alfred Yung, Jasti S. Rao, Houston, TX, USA.

OBJECTIVE: To determine the role of p16 gene in suppression of invasiveness of glioma tumors.

BACKGROUND: The diffuse invasion of the brain parenchyma by malignant astrocytomas is one of the most important barriers to successful therapy. Better understanding of the regulators of the proliferation and invasion of these tumors may help to improve current therapy. Alterations of the tumor suppressor gene p16 are among the most frequent events in high-grade gliomas. We have previously reported that restoration of the wild-type p16 function induced growth arrest and modified the transformed phenotype of glioma cells, suggesting that p16 constitutes a suitable target for gene therapy of gliomas. Studies of the role of p16 on invasiveness have not been reported yet in any cancer.

DESIGN/METHODS: We used a recombinant replication deficient adenovirus to infect and transduce high levels of p16 protein to null-p16 human glioma cells. Invasiveness was tested in two models: Matrigel-coated transwell inserts and co-culture of tumor spheroids and fetal rat brain aggregates. Briefly, fluorescent stained tumor spheroids and brain cell aggregates were transferred to individual agar-coated wells. With the help of a stereomicroscope spheroids and fetal brain were placed in close contact to each other. Serial optical sections were obtained from the surface to the center of the co-cultures by confocal Laser Scanning Microscopy. Detection of the different fluorescent staining of tumor spheroids and fetal brain aggregates was performed using Argon and Helium/Neon lasers. In addition, analysis of the expression of 72-KDa matrix metalloprotease (MMP-2) by gelatin zymography was performed.

RESULTS: Matrigel invasion assays showed that the SNB19 and U-251 MG cells treated with p16 had significant reduced invasion. Similarly, invasion of SNB19 cells expressing exogenous p16 showed reduced invasion into fetal rat brain aggregates during a 72 h time period, compared to parental SNB19 and vector infected cells. The expression of MMP-2 in SNB19 cells infected with p16 was significantly reduced compared to parental SNB19 and vector infected cells.

CONCLUSION: Restoration of wild-type p16 activity into null-p16 glioma cells, significantly inhibited the process of tumor invasion. Our data document a novel function of p16 and enhance p16 as a gene therapy target.

VI3.003
4:15 PM

Crossroads of Death and Life Signals in Gliomas: p53 and p21 Interactions

Candelaria Gomez-Manzano, Juan Fueyo, Athanassios P. Kyritsis, Victor A. Levin, W.K. Alfred Yung, Houston, TX, USA.

OBJECTIVE: To determine the timing of the cellular and molecular events that preceded and regulated p53-mediated apoptosis in human glioma cells.

BACKGROUND: Prevention of neoplasia requires accurate balance between cellular proliferation and apoptosis. The p53 tumor suppressor gene is mutated in 50% of gliomas and constitutes an early genetic event, suggesting that p53 is involved in glioma genesis. In a previous report we demonstrated that adenovirus-mediated wild-type p53 transfer induced apoptosis in mutant p53 glioma cells indicating that p53 is a suitable target for gene therapy strategy of gliomas. Recently it has been reported that induced expression of the p21 gene prevented differentiating cells from undergoing apoptosis. Since understanding the functional relationship between p53 and p53-related genes, like p21, may help to design more rational treatments for brain tumors we undertook this study to ascertain whether p21 may modulate p53-mediated apoptosis.

DESIGN/METHODS: In this system we used replication deficient adenovirus constructs carrying either p53 or p21 cDNAs as vehicles for gene transfer. Flow cytometry techniques were used to examined the cell cycle phenotype of the treated glioma cells. Immunohistochemistry and western blot were performed to assess the expression of both, the exogenous and the induced genes. Apoptosis was studied by morphological and quantitative methods, including acridine orange nuclear staining and determination of the sub-G1 population of cells.

RESULTS: After the transfer of exogenous wild-type p53, induction of p21, G2 arrest, elevation of bax and apoptosis were consecutive events. Interestingly, expression of exogenous p21 induced arrest of glioma cells in G2 phase. To analyze p53 and p21 interactions in the process of apoptosis, p21 and p53 genes were coexpressed. Previous transfer of the p21 gene resulted in significant protection from p53-mediated apoptosis. Thus, cul-